CHANGES IN THE IMMOBILE AQUEOUS PHASE OF CELLULOSE DURING CHROMATOGRAMS FORMED WITH ABSOLUTE ETHANOL

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For a chromatographic system operated at near-equilibrium conditions, the fraction of solute molecules in the mobile phase at any time in any infinitesimal segment, dx, of the migration path is given by[1,2]

$$R_i = \frac{1}{1 + \alpha_i(A_L/A_M) + \beta_i(A_S/A_M)} \quad (1)$$

where α_i is the ratio of the concentration of the ith solute in the stationary liquid to that in the mobile fluid, β_i is the ratio of the solute concentration adsorbed on the solid support to that in the mobile phase, A_L is the cross-sectional area of the immobile liquid, A_S is the "cross-sectional area" of the solid support, *i.e.*, it is some measure of the extent of the solid surface, and A_M is the cross-section of the mobile fluid. The term $\alpha_i(A_L/A_M)$ measures solute retention by the partitioning liquid and $\beta_i(A_S/A_M)$ measures solute retention by the partitioner support. If β_i is very small, *i.e.*, the support is essentially inactive, or if $A_L \gg A_S$ (*cf.* ref. 3) then $\beta_i(A_S/A_M)$ is negligible and

$$R_i = \frac{1}{1 + \alpha_i(A_L/A_M)} \quad (2)$$

If α_i, A_L, A_M, and the velocity of the mobile fluid are constant in the direction of solvent flow (x-direction), then for liquid-liquid chromatograms, $R_i = R_{Fi}$ where R_{Fi} is the ratio of the distance moved by the center of the zone of the ith solute to the distance moved by the mobile fluid front. Insufficient consideration has been given to the dependency of A_L and A_M on the coordinate x. GIDDINGS, STEWART AND RUOFF[4] have shown that A_M is not constant along the migration path in paper chromatography. This leads to a considerable dependency of R_F upon x for certain R_F-values. In gas–liquid chromatography, A_L may be reduced at the column inlet by evaporation of the liquid partitioner[5–7]. Similarly for liquid–liquid systems, if the mobile phase is not saturated with the liquid acting as the immobile phase, it will extract the latter from the support until saturated. Not only is A_L a function of the x-coordinate but also, in the region of partitioner loss, A_L may be so reduced that the solid adsorption term of eqn. (1) cannot be ignored while further along the path, eqn. (2) is appropriate. It may also be that α_i has one value in the region for saturated mobile phase and a different value in the region of unsaturated carrier. Generally this problem can be

avoided when the mobile and immobile liquids used are immiscible in the bulk by saturating each with the other before forming the chromatogram. Nonequilibrium between these two phases has led to anomalous results, *e.g.*, the appearance of two zones for a single solute[8].

A problem arises when the mobile phase is a liquid which is completely miscible with the liquid of the immobile phase[9–12]. The equilibrium concentration of partitioner in the mobile phase is not known and if there is nonequilibrium, A_L will not be constant.

The system selected for study here was paper, where the immobile phase is water, and a mobile phase of absolute ethanol. Because of the difficulty of analyzing paper for its water content, we elected to measure it in the eluant fractions much as one seeks partitioner in the effluent gas in gas–liquid chromatography.

EXPERIMENTAL

Gas chromatographic analysis

The problem was the analysis of 0.2 ml eluant fractions from paper and cellulose pulp column chromatograms where the amount of water in the ethanol was 10% or less. Gas chromatography seemed the best method for fast, simple, and duplicate analysis.

The equipment was a Cenco No. 70130 Vapor Phase Analyzer (Central Scientific Co., Chicago, Ill., U.S.A.) with a thermal conductivity cell. The machine had nine sensitivity settings by which the response, and hence the height of the concentration profile, could be adjusted. Column, detector, and sample injection unit were all at the same temperature. Concentration profiles were recorded on a Leeds and Northrup Speedomax Model S, variable range, variable sensitivity recorder of one second response time and 30 in./h chart speed. Driving pressure was measured by a mercury manometer at the column inlet and flowrate by a soap-film flowmeter at the outlet. Sample introduction was by a 100 μl Hamilton syringe.

A difficulty with any analysis of a binary mixture where the proportion of one component to the other is very small (trace analysis) is that the peak of the principal constituent is very much larger than that of the other. There is considerable error in measuring the small peak areas[13–15]. The equipment lacked an automatic attenuation device to reduce the response of the detector in proportion to the signal. Attenuation was accomplished by manual adjustment. The alcohol peak was kept on scale by using a large millivolt range on the recorder and a low sensitivity setting on the gas chromatograph. After the alcohol peak had passed, the millivolt range was reduced on the recorder, and the sensitivity on the gas chromatograph was increased which magnified the following water peak. This procedure required a sufficient difference in retention times of the ethanol and water so that the adjustment could be made and a reliable base line established. The retention times themselves could not be too large since this broadened and flattened the water peak so that it was indistinguishable from the background circuit noise.

The column used was 3 ft. of coiled 0.25 in. O.D. copper tubing packed with 12.1 g of 30–50 mesh of Neutraport-T, a fluorocarbon (Micro-Tek Instruments Inc., Baton Rouge, La., U.S.A.), bearing THEED (tetrahydroxy-ethylenediamine)[16,17] (Applied Science Laboratory, State College, Pa., U.S.A.) prepared by evaporation of an acetone solution of the partitioner while in contact with the support. Repeated

extraction of the packing indicated a 10.5 % liquid load. The column was conditioned by passing helium through it at 23.3 ml/min for 7 h at 100°. THEED is very hygroscopic. A water peak always appeared on the record whenever the machine was started. Before every series of analyses, the machine was allowed to run at the operating conditions until this water peak was eluted and a constant base line established. A driving pressure of 10 cm Hg gave a consistent carrier flow of 30 ml/min at 84°. The retention times of ethanol and water were 5.0 min and 24 min respectively and permitted the adjustment of the machine between peaks without introducing appreciable tailing or peak broadening. Tailing was never completely eliminated. The retention times varied slightly with sample size. After nine months of use, the retention time of ethanol was reduced to about 3.5 min while that of water was about 18 min, which is the type of change that one would expect with loss and redistribution of partitioner[5-7].

Samples of known composition were prepared from absolute ethanol (U.S. Industrial Chemicals Co., New York, U.S.A.) and water. Peak areas were measured with a planimeter and the average of three measurements used to compute the per cent area of the water and ethanol peaks. No adjustment was made for the attenuation. A least squares line was fitted to the data points for the variation of area per cent *vs.* water content for the prepared samples of known composition from 1.16 to 10.66 % weight by water (16 data points, 20 μl samples) and 0.59 to 2.28 % (12 data points, 70 μl samples) using both weight per cent and mole per cent and a linear regression coefficient[18] computed in each case. Both plots were slightly nonlinear with virtually identical regression coefficients (0.9869 and 0.9861 respectively). We used weight per cent since it reduced the computations. The deviations of the area per cents from the mean were computed for each sample and their squares pooled to calculate a standard deviation. An average estimate of the error based on twice the standard deviation is 0.45 % water for the range of 0.59 to 10.66 % water. Samples containing 0.5 % water gave small but measurable peaks. This was the lower limit of this method of analysis. For samples of 0.35 % water or less, peaks were not detected. Between these extremes, the water content of samples was estimated by visual comparison of the tracing with those of standards. Samples of the same composition and differing in size by less than 20 % gave per cent areas which varied within experimental error. Outside of this limit, peak area per cents were detectable as dependent upon the sample size.

Because of the change in the properties of the column with use as indicated by the change in retention volume, calibrations were interspersed with analyses. There was insufficient change in the standard curves to warrant the construction of new calibration curves during the collection of pertinent data.

Sample storage

Sample collection tubes were fastened firmly to the outlet of the chromatographic system by means of a tight fitting cork with a small vent on its side and collections were made at room temperature. Tubes, with their solvent fractions, were tightly stoppered with corks and stored in a glass bottle with a tightly fitting screw cap. This bottle was placed in a desiccator over calcium chloride until analyzed. Even with this, the water content of the stored samples increased as will be pointed out later.

Cellulose pulp columns

Ethanol was added to a 22 × 2.2 O.D. cm chromatographic tube from a separatory funnel protected from atmospheric moisture by a drying tube containing calcium chloride. Collection was made in carefully dried test tubes, calibrated for 0.2 ml. Fig. 1 shows the percentage water as a function of total eluant volume for a column

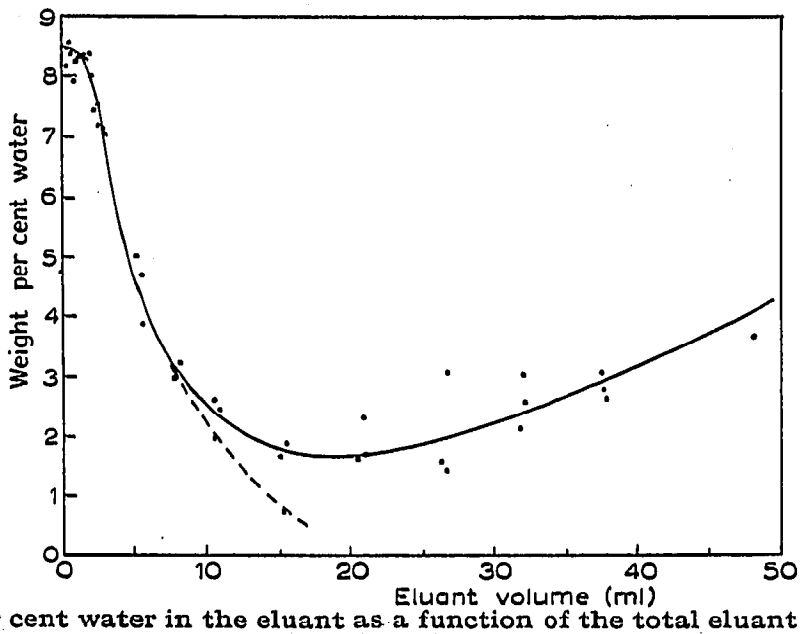

Fig. 1. Weight per cent water in the eluant as a function of the total eluant volume for the 17 cm column of cellulose pulp.

of cellulose pulp (Whatman Cellulose Powder, Standard Grade) 17 cm in length and weighing 18.21 g. Fig. 2 shows the data for a column, 9 cm long, weighing 10.72 g. Analysis of the alcohol in the reservoir on completion of the chromatograms did not detect water.

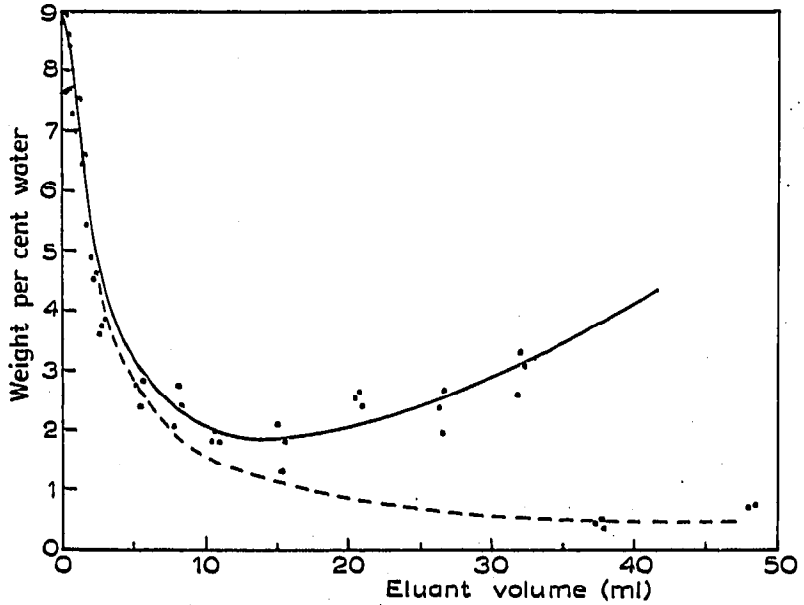

Fig. 2. Weight per cent in the eluant as a function of the total eluant volume for the 9 cm column of cellulose pulp.

Both Figs. 1 and 2 show about 9 % water in the first eluant fractions. It was not thought that the increasing amount of water present in later eluant fractions had meaning in terms of the chromatographic process but was due to contamination of the samples in storage. Fig. 1 represents 92 individual analyses and Fig. 2 represents 103 analyses. As rapid as gas chromatography was as an analytical method, a period of over a week elapsed before the last samples were analyzed and there was ample time for contamination. Fig. 1 shows a sample of 15 ml total eluant volume where the water content was much lower than the others. This particular sample was analyzed much earlier than the others in this region. Fig. 2 shows fractions at 37 and 48 ml total eluant volume which were also among the first analyses. Their water content was about 0.5 % while those at 20 to 30 ml total eluant volume showed 2.5 to 3 % water. For these reasons, the dotted lines in Figs. 1 and 2 are probably better estimates of the water content of successive eluant samples than are the solid lines.

Each point of Figs. 1 and 2 represents the mean of from two to four analyses of an eluant fraction. A deviation from the mean was computed for the analysis of each 0.2 ml fraction and the squares of these combined to calculate a pooled standard deviation corresponding to a precision of 0.60 % at the 95 % confidence limit.

Paper strip chromatography

The chamber for the paper chromatography consisted of a glass pipe 19.4 cm in length and 7.0 cm I.D. stoppered at both ends. An aluminum cradle hung from the top stopper and held a glass solvent boat. Solvent was added to the boat through a hole in the stopper which was closed during the experiments. The lower stopper held a thistle tube which collected the drippings from the paper and delivered them to calibrated test tubes.

Whatman Filter Paper No. 1 was cut into strips measuring 22.6 × 4.1 cm. The solvent passed through about 19.1 cm of paper in its journey from the solvent surface in the boat to the end of the strip. A typical strip weighed 0.803 g, which meant that the solvent passed through 0.678 g of cellulose. Before use the chamber was rinsed twice with absolute ethanol and 25 ml of ethanol was placed in the bottom of the chamber so that an alcohol saturated atmosphere was established in the chamber.

All experiments were performed at room temperature (26–28°). The papers were treated in several ways. (1) The paper strip was suspended in the chamber overnight in contact with ethanol vapors. The solvent of absolute ethanol reached the bottom of the strip in about two hours and 1.5 to 1.8 h were required to collect 0.2 ml of eluant. Analysis showed no detectable water. (2) The strip was placed in the chamber and the solvent flow was started immediately. Analysis showed 0.35 % or less water in one experiment and 0.50 % water in another. A detectable amount of water was present in the second case. (3) The paper strip was allowed to stand overnight in an atmosphere saturated with water, dried at room temperature for 5 h, hung overnight in ethanol vapors in the chromatographic chamber, and the experiment performed. Analysis gave no detectable water. (4) The paper strip was hung overnight in a water saturated atmosphere, dried at room temperature for 5 h, placed in the chromatographic chamber and the experiment begun immediately. Analysis gave 0.42 and 0.43 % water.

Discussion

For the gas chromatographic analysis reported here, water can be analyzed in solution in ethanol in the range of 0.5 to 10 % water with an average error of 0.6 % water. Water in a concentration as low as 0.35 % can be detected but between 0.35 and 0.50 %, the analysis is only approximate.

Absolute ethanol is capable of removing water from cellulose pulp to reduce the amount of the immobile phase and perhaps alter the chromatographic properties of the system, *i.e.*, introduce retention by the support. This water appears in the first eluant from the column to give a sharp change in the composition of the solvent flowing from the column. The concentration of water (about 9 %) is independent of the length of the column and the quantity of packing. The total amount of water in the eluant depends upon the amount of cellulose pulp. The area under the dotted curve above 1 % water content for Fig. 1 is 1.6 times the same area for Fig. 2. These areas are measures of the total amount of water in the eluant. The ratio of the weight of cellulose pulp in the two columns was 1.7 which shows a good correlation between the water content of the eluant and the weight of the packing materials. If these same areas are used to calculate the total amount of water removed per gram of cellulose, the result for the 17 cm column is 0.024 g and for the 9 cm column is 0.026 g to give an average of 0.025 g. If cellulose pulp is assumed to have normally 10 % by weight water[19] the ethanol removed 25 % of this bound solvent.

Using the data for the cellulose powder, the ethanol, in passing through the 0.678 g of cellulose of the paper strip, should have removed 0.017 g of water. If this appeared in the first 0.2 ml fraction, the percentage water would have been 8.5 %. This was not the case. Water could not be detected in the eluant from papers allowed to stand in vapors of ethanol overnight and was just detectable in eluants from papers used immediately after placing them in the ethanol atmosphere. This was true whether the paper was presaturated with water or not. The absence of water in the eluant does not mean that the paper was not dehydrated. Such a hypothesis would rest on the assumption that the cellulose of the column was different from the cellulose of the paper. The explanation must lie in the presence of ethanol vapors in the paper chromatographic chamber but which were not present in the column. As shown by the columns, the ethanol extracts water from the paper as it migrates down the paper. This solution, because of its water content, exerts a higher vapor pressure than ethanol (95 % ethanol boils at 78.15° while ethanol boils at 78.3°) and will evaporate into the atmosphere to remove water and reduce the concentration of water in the mobile phase. This can be seen by considering the fractional distillation of a solution of water content less than 5 %. The composition of the vapor approaches that of the azeotrope while the composition of the residue approaches pure ethanol. There is sufficient time for this to occur since very nearly 2 h were required for the ethanol to reach the end of the paper and nearly 1.5 h was required for a drop to form.

Apparently water is also lost from the immobile phase by a similar process if the paper is allowed to stand in the ethanol atmosphere because no water was found in the eluant under these circumstances whereas a trace of water was found where there was no equilibration.

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SUMMARY

Developers which are miscible with water are capable of removing some of the immobile aqueous phase from cellulose if they are initially anhydrous. Eluant fractions from cellulose columns developed with absolute ethanol showed a water content of about 9 % (w/w) and a total content equivalent to removal of about 25 % of the immobile liquid phase. Eluants from paper chromatograms showed no water probably due to evaporation of the solution from the paper in the chamber. Analysis was by gas chromatography using THEED supported by a fluorocarbon. The average error was 0.6 % water in the range 0.5 to 10 % water. Water could not be detected below 0.35 %.

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